Case Report Rapport de cas

Effect of maternal ketoacidosis on the ovine fetus

Diego E. Gomez, Shikha Kuthiala, Hai L. Liu, Daniel L. Durosier, Mingju Cao, Patrick Burns, André Desrochers, Gilles Fecteau, Martin G. Frasch

Abstract — Ketoacidosis during pregnancy carries significant risk of intrauterine fetal demise, but little is known about the impact of ketoacids on the ovine fetus. We report a case series of maternal ketoacidosis in ewes. Maternal ketoacidosis may result in biochemical and acid-base fetal abnormalities associated with changes in feto-placental unit perfusion.

Résumé — Effet de l'acidocétose maternelle sur un fœtus ovin. L'acidocétose durant la gestation comporte un risque important de mortalité intra-utérine du fœtus, mais on connaît peu de choses à propos de l'impact des acides cétoniques sur le fœtus ovin. Nous signalons une série de cas d'acidocétose maternelle chez les brebis. L'acidocétose maternelle peut provoquer des anomalies biochimiques et acides avec des changements dans la perfusion de l'unité fœto-placentaire.

(Traduit par Isabelle Vallières)

Can Vet J 2015;56:863–866

Case description

Four pregnant ewes (126 to 127 d of gestation) were presented to the Centre Hospitalier Universitaire Vétérinaire (CHUV) of the Université de Montréal in Saint-Hyacinthe for evaluation. The ewes were part of a research project in chronically instrumented non-anesthetized fetal sheep (1). The research protocol was approved by the Committee on Ethics of Animal Experiments of the Université de Montréal (protocol number 10-rech-1560). The preparation for surgery implied a change in immediate environment (from the farm of origin to the research unit) as well as a period of fasting to allow general anesthesia. Seven days before surgery, the ewes were transported to the experimental facility. The ewes were fasted for 36 h and water was withheld 18 h before surgery. Intravenous access was achieved using a single-lumen catheter via the jugular vein. The ewes were sedated with acepromazine (Acevet 25; Vétoquinol, Lavaltrie, Quebec), 2 mg IV approximately 30 min before the induction of anesthesia. Diazepam (Diazepam INJ USP; Sandoz Canada, Boucherville, Quebec), 0.03 mg/kg body weight (BW), ketamine (Vetalar; ketamine hydrochloride injection USB; Bioniche Animal Health Canada, Belleville, Ontario), 5 mg/kg BW, and propofol (Propoflo; Abbott Animal Health, Abbott Park, Illinois, USA), 1 mg/kg BW, were given intravenously to induce general anesthesia.

A midline incision was made in the lower abdominal wall and the uterus was palpated to determine the fetal position. The upper body of the fetus was exteriorized through an incision in the uterine wall. Polyvinyl catheters about 200 cm long constructed from micro medical tubing (Scientific Commodities, Lake Havasu, Arizona, USA) (inner diameter 0.72 mm × outer diameter 1.22 mm) were placed in the fetal right and left brachiocephalic arteries, the cephalic vein, and the amniotic cavity. The total duration of the procedure was approximately 2 h. Trimethoprim-sulfadoxine (Borgal; Intervet Canada, Kirkland, Quebec), 25 mg/kg BW, IV, was given to the ewe and the fetus and ampicillin (Ampicillin sodium; Novopharm. Toronto, Ontario) was deposited in the amniotic cavity. The catheters were exteriorized through the maternal flank and secured to the back of the ewes in a plastic pouch. The ewes were returned to the metabolic cage, where they could stand, lie, and eat *ad libitum.* Approximately 2 d after surgery the ewes appeared depressed, anorectic, and weak, and were presented to the CHUV for diagnosis and treatment.

Department of Health Management, Atlantic Veterinary College, University of Prince Edward Island, Charlottetown, Prince Edward Island (Gomez); Faculté de Médecine Vétérinaire, Université de Montréal, St. Hyacinthe, Québec (Burns, Desrochers, Fecteau, Frasch); Department of Obstetrics-Gynecology, Faculté de Médecine, Université de Montréal, CHU Sainte-Justine Research Center, Québec (Kuthiala, Liu, Durosier, Cao, Frasch).

Address all correspondence to Dr. Diego Gomez; e-mail: dgomez@upei.ca

Presented in part as an oral presentation at the 2013 at the Dorothy Havemeyer Neonatal Septicemia Workshop VI, Saugerties, New York, USA.

Table 1. Maternal venous blood gas and fetal arterial blood gas and plasma concentration of glucose, electrolytes, L-lactate, and β-hydroxybutyrate of 4 ewes/fetuses with ketoacidosis

Parameter	Ewe 1/Fetus 1	Ewe 2/Fetus 2	Ewe 3/Fetus 3	Ewe 4/Fetus 4	Maternal RR	Fetal RR
pH	7.27/7.37	7.44/7.35	7.40/7.35	7.43/7.31	7.45 ± 0.07	7.331 ± 0.004
P_vCO_2/P_aCO_2 (mmHg)	33/41	39/48	38/47	39/57	36.8 ± 8	48.5 ± 0.3
P_vO_2/P_aO_2 (mmHg)	66/22	57/25	45/20	56/18	75 ± 24	24 ± 0.3
HCO_3^- (mmol/L)	14/22	25/26	22/24	24/27	26 ± 2	25 ± 0.2
AG (mmol/L)	20/18	19/15	22/16	14/12	6–17	6–17
Glucose (mmol/L)	30/12	43/15	32/11	100/28	56 ± 3	16 ± 2
Na^+ (mmol/L)	148/141	148/142	149/141	149/141	145 ± 3	139 ± 0.4
K^+ (mmol/L)	2.9/4.2	3.4/3.7	4.2/4.6	3.7/3.8	4 ± 0.4	3.9 ± 0.7
Cl^- (mmol/L)	109/105	108/105	109/106	115/107	105 ± 4	103 ± 1.4
Ca^{2+} (mmol/L)	1.1/1.23	1.07/1.34	1.18/1.42	1/1.3	1.2 ± 0.2	1.4 ± 0.1
βHB^- (mmol/L)	6.6/0.3	3.9/0.1	5/0.1	1.3/0	0.4–1.3	0.02 ± 0.01
$Lactate^-$ (mmol/L)	0.9/1.7	0.5/1.1	0.9/1.2	0.6/2	1.6 ± 0.9	0.85 ± 0.02

P_vCO_2 — partial venous pressure of carbon dioxide; P_aCO_2 — partial arterial pressure of carbon dioxide; HCO_3^- — bicarbonate; AG — anion gap; βHB^- — β-hydroxybutyrate; RR — reference range.

Physical examination and diagnostic test results

On admission the ewes had a body condition score of 3 to 3.25/5 (2). All 4 ewes appeared depressed, weak, and were reluctant to move. Rectal temperature, heart rate, and respiratory rate were within normal limits. All 4 ewes had regular peripheral pulses, cool distal extremities, slightly sunken eyes, and skin tenting of 2 s. The mucous membranes were pink and tacky. Capillary refill was < 2 s and jugular refill was normal. Dehydration was estimated to be 5%. Auscultation of the lungs was unremarkable. Auscultation of the left side of the abdomen revealed decreased frequency and amplitude of ruminal contraction. Abdominal ballottement was unremarkable. No specific neurologic deficits were detected except for a profound depression.

The initial diagnostic evaluation included venous blood gas analysis (VBGA), plasma concentration of glucose, electrolytes, and β-hydroxybutyrate (βHB). Blood gas, glucose, and electrolytes were determined using an ABL800 blood gas analyzer (Radiometer Canada, London, Ontario) that was set for sheep, adjusted by body temperature at 39°C. The βHB levels were tested using Precision Xtra Blood Ketone Monitoring System (Abbot Diabetes Care, Alameda, California, USA). Abnormalities included moderate hypoglycemia and severe anion gap (AG) acidosis in ewes 1, 2, 3, marked hyperglycemia in ewe 4, severe increase in plasma βHB in all 4 ewes, and moderate hypokalemia in ewes 1 and 2 (Table 1) (3). These abnormal findings prompted analysis of fetal arterial blood gas (ABGA) and determination of fetal plasma βHB. The analyses revealed mild to moderate hyper-L-lactatemia (3) in all 4 fetuses and a mild to moderate decrease in the arterial P_aO_2 (3) (fetuses 1, 3, 4) (Table 1). Fetal plasma pH and βHB were within normal limits. Based on the history, clinical and laboratory findings, a diagnosis of ketoacidosis was made for all 4 ewes.

Treatment and outcome

Treatment focused on management of acid-base and electrolyte abnormalities. Correction of dehydration was accomplished by IV administration of crystalloid Lactate Ringer's solution (Baxter Corporation, Mississauga, Ontario), 100 mL/kg BW per day, dextrose 8 mg/kg BW per hour, calcium borogluconate (Calcium Borogluconate 23%; Vétoquinol), 6 mg/kg BW per hour and potassium chloride (Potassium chloride USP; Hospira, Montreal, Quebec), 0.04 mmol/kg BW per hour. After the first 24 h, hydration status was normalized. Appetite, rumen contraction, and gastrointestinal (GI) motility improved markedly. The VBGA of ewes 2 and 3 were unremarkable but a mild to moderate AG acidosis [pH 7.2; reference range (RR): 7.45 ± 0.07 and AG 21 mmol/L; RR: 6 to 17 mmol/L] was detected in ewe 1. Plasma K^+ of ewe 3 was within the normal range but remained below the RR in ewes 1 and 2 (3.9 mmol/L and 3.4 mmol/L, respectively; RR: 4.6 to 6.5 mmol/L). Maternal concentration of βHB decreased mildly in ewe 1 (βHB 4.1 mmol/L; RR: 0.38 to 1.32 mmol/L), whereas it was within the normal limits in ewes 2 (1.3 mmol/L, 3 (0.9 mmol/L), and 4 (0.4 mmol/L) (3). Therapy with intravenous fluid and dextrose was discontinued in ewes 2 and 3.

Forty-eight hours after admission, the attitude, appetite, and GI motility of ewe 1 normalized. Plasma βHB approached the normal range and VBGA were within the normal limits (pH 7.43, HCO_3^- 26 mmol/L, RR: 25.8 ± 2 mmol/L, AG 17 mmol/L). The fetal ABGA and plasma electrolytes of all 4 fetuses were within the reference ranges (3). Therapy with intravenous fluid and dextrose was discontinued in ewe 1. Due to the good response to the therapy and correction of fluid, electrolyte and acid-base disturbances the ewes were returned to the experimental facility. All 4 fetuses were viable at the end of the study. Birth weights ranged from 2.6 kg to 3.4 kg. The fetuses were euthanized after birth as per the research Ethics of Animal Experiments protocol.

Discussion

The effect of maternal ketoacidosis on the fetuses has received little attention in veterinary medicine, especially in ewes with pregnancy toxemia. To the authors' knowledge this is the first report describing the effect of ewe's ketoacidosis on fetal health.

Glucose is quantitatively the most important nutrient crossing the placenta, followed by amino acids (4), and the development of the fetus directly depends on their continuous availability. About 60% of fetal growth takes place in the last part of gestation, when approximately 33% to 36% of the circulating glucose of the ewe is directed into the fetoplacental unit (5). As

lambing approaches the plasma concentration of glucose declines and concentration of ketone bodies (KB) increases slightly, but the decrease in glucose is relatively modest compared to the increase in KB (6). In the third trimester of pregnancy plasma KB greatly increases under fasting conditions or negative energy balance (7) as a consequence of enhanced adipose tissue lipolysis, which accelerates the delivery of non-essential fatty acids (NEFA) to the liver and enhances ketogenesis (8). In the late gestation, ewe βHB utilization is reduced and this reduction is greater in twin- than in single-bearing ewes, which leads to increased plasma βHB concentrations (9). Furthermore, endogenous glucose production in pregnant ewes is depressed by βHB, but there is no effect on glucose utilization (10). Although the pathogenesis of pregnancy toxemia in ewes is not completely understood, the disease is associated with a decline in plane of nutrition leading to a negative energy balance sufficient to produce hypoglycemia, hyperketonemia and clinical signs (11).

The fetal lamb utilizes glucose to account for, at most, one half of its oxygen consumption (12). However in starved ewes, only approximately 17% of the aerobic needs of the fetus could be accounted for by glucose acquired via the umbilical vein (12). Fetal survival in prolonged starvation indicates that the fetus can compensate for this decrease in glucose levels by increasing the utilization of other substrates (12,13). In any condition of low glucose availability such as starvation or insulin deficiency, there is a deficit of pyruvate entering the citric acid cycle as glycogen stores are depleted (14). Therefore alternative sources of energy have to be used, and these are provided by generation of acetyl CoA from bioxidation of fatty acids. The quantity of acetyl CoA produced may exceed the capacity of the citric acid cycle, leading to the formation of βHB, acetoacetate, and acetone (14). While ketogenesis is not active in the fetus (15), in human fetal plasma, the same level of βHB can be reached as in the mother, since it easily crosses the placenta (16). Ketone bodies might be used as an alternative energy source for many tissues, including the placenta (11). Ketone bodies can also be used as a fuel source, as they can serve as a substrate for myelin and lipid synthesis in the developing brain (17). Brain uptake of βHB was studied during intravenous infusions in catheterized fetal sheep at 135- to 141-days gestation (18). That study indicated that the near term fetal sheep brain appears to take up βHB when arterial blood glucose is low (18). Studies in rats also suggest that ketones confer neuroprotection during ischemia and traumatic brain injury (19–22). However, in severe ketoacidosis the human fetus can become acidotic from βHB that crosses the placenta (23). In humans, maternal hyperketonemia, with or without acidosis, is associated with increased stillbirth, an increase in incidence of congenital anomalies, and impaired neurophysiological development in the infant (23).

The impact of maternal-derived βHB on fetal acid-base balance in the ovine fetuses in this report was insignificant. This suggests that the amount of βHB crossing the placenta may be lower in ruminants than in humans. The transfer of ketone bodies occurs by simple diffusion or by low-specificity carrier-mediated processes and differs among species, being much lower in ruminants than in nonruminants (16). This causes major differences in the materno-fetal gradients for ketone bodies, which is above 10 in ewes (24) and 2 in humans (8). Although maternal ketonemia of multiple-fetus pregnancies is accompanied by increased uterine uptake of βHB, little or no βHB is transported to the fetus, so ketone bodies then become an important energy source for the placenta, but not for the fetuses (25). Our findings are consistent with reports that the amount of βHB crossing the placenta is much lower in ruminants than in nonruminant species. The contribution of ketone bodies to fetal oxidative metabolism is only 2% to 3% in ewes even during maternal starvation (24). These findings lead us to hypothesize that the type of placentation in the ewes (syndesmochorial placentation) prevents the transfer of maternal-derived βHB to the fetal circulation and therefore its impact on fetal acid-base is minimal.

Severe ketoacidosis in the ewe may impact the health of the fetus in 2 ways: starvation of the fetus (energy deficiency), but not necessarily the placenta which can utilize the ketone bodies present at the maternal-fetal cotyledonary junction; and poor perfusion of the feto-placental unit (14). It is well-recognized that human ketoacidosis can result in intrauterine death, with mortality rates from 9% to 35% (14). Severe maternal ketoacidosis reduces uterine blood flow leading to decreased perfusion of the feto-placental unit, reduced P_aO_2, transient abnormal fetal umbilical and cerebral blood flow, and increased blood lactate levels and fetal acidosis (26,27). L-lactate does not effectively cross the maternal-fetal placental junction (28). Therefore, elevated L-lactate in the ewe is not likely responsible for increases in the fetus (29). Elevated fetal L-lactate concentrations and decreased P_aO_2 in the fetuses reported here are most likely due to reduced perfusion of the feto-placental unit (30). Rapid recognition of the clinical signs and medical intervention aimed to correct disturbances of free fluid, electrolyte and acid-base imbalances, negative energy balance, and limit fat mobilization may have led to a rapid resolution of the ketoacidosis in the ewes and minimized the impact of hypoperfusion on the fetus' hemodynamic state.

We conclude that increased maternal plasma βHB concentrations led to maternal acidosis in the pregnant ewes. The impact of maternal βHB on fetal acid-base balance in the ovine fetus was insignificant, but ketoacidosis resulted in a mild increase in fetal L-lactate concentration and decreased P_aO_2, likely due to changes in feto-placental unit perfusion. CVJ

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